functional correction of the HD phenotype. Even stronger protection against HD pathology may be afforded by optimization of vector pseudotype and intrabody expression level.

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Intrabody patent application pending (NYS DOH/Abgenix)

49. AAV Vector-Mediated Gene Delivery of Aromatic L-Amino Acid Decarboxylase Restored L-DOPA Efficacy in a Primate Bilateral Model of Parkinson's Disease

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Parkinson's disease (PD) is a progressive movement disorder marked by selective degeneration of dopaminergic neurons of the substantia nigra and severe decrease in the dopamine (DA) content of the striatum. Replacement of DA is important to alleviate the motor symptoms of the disease. The DA precursor, L-3,4-dihydroxyphenylalanine (L-dopa), which is converted to DA by aromatic L-amino acid decarboxylase (AADC), provides clinical benefit to virtually all patients and has been mainstay in pharmacotherapy for PD. However, as the disease progresses, severe loss of dopaminergic nerve terminals is associated with an 80-95% depletion of striatal AADC activity resulting in loss of L-dopa efficacy. We demonstrated previously that AAV vector-mediated gene transfer of three DA-synthesizing enzymes, namely tyrosine hydroxylase, AADC, and GTP cyclohydrolase I, resulted in behavioral recovery in animal models of PD with efficient and long-term transduction of striatal neurons. In the present experiments, we investigated the therapeutic potential of the combination therapy, gene transfer of AADC with oral administration of L-dopa, in a primate bilateral model of PD. Cynomolgus macaques (*Macaca fascicularis*) were chronically treated with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) to make bilateral striatal lesions. Animals became behaviorally stable and motor symptoms did not ameliorate by the administration of L-dopa with peripheral AADC inhibitor. Then, adeno-associated virus (AAV) vector expressing AADC was injected into the unilateral putamen stereotaxically. Expression of AADC in the unilateral putamen resulted in remarkable improvement in manual dexterity on the contralateral to the AAV-AADC-injected side after the oral administration of L-dopa with peripheral AADC inhibitor. Fine motor task consists of capturing a piece of an apple improved on the contralateral hand with L-dopa. Amelioration of motor symptoms persisted about four hours each time after the oral intake of L-dopa. This duration of efficacy corresponds to the effective blood concentrations of the drug. Positron emission tomography showed remarkable and persistent increase of [11C]-L-dopa uptake in the AAV-AADC injected putamen. Gene transfer of AADC alone in combination with oral administration of L-dopa could be a shortcut to starting clinical trials of gene therapy for PD. With this method, patients still need to take L-dopa for the control of PD symptoms, but DA production can be regulated by the dose of L-dopa .

50. AAV-Mediated Expression and Secretion of ADNF-9 Protects Substantia Nigra Neurons from 6-OHDA Damage

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Activity dependent neurotropic factor (ADNF) occurs endogenously in astrocytes, and both ADNF and the fragment, ADNF-9, exert potent neuroprotective properties (Brenneman et al.,JPET:285,619,1998). Recently, Haberman et al. (Nat. Med.9:1076,2003) showed that the fibronectin secretory signal sequence (FIB) could be used to constitutively secrete an active gene product from neurons transduced by adeno-associated virus vectors(AAV) *in vivo*. Given the extraordinary potency of ADNF (femtomolar range), we conducted a study to determine if linking the FIB to the ADNF-9 coding sequence would prove sufficient to protect substantia nigra neurons from striatal infusions of the neurotoxin, 6-OHDA. Rats first received a 2 microliter unilateral striatal infusion of AAV-FIB-ADNF and one week later, received two unilateral striatal infusions of 6-OHDA (3.5 micrograms/ 1 microliter per infusion). Two weeks later, the rats were perfused, and the presence of tyrosine hydroxylase (TH) was determined immunohistochemically. In the striatum 6-OHDA treatment caused a significant loss of TH containing terminals both in the control and AAV-FIB-ADNF treated rats. In the substantia nigra, the control 6-OHDA rats exhibited a 47±5% decrease in TH positive cells compared to the untreated side. However, no decrease in TH containing cells was observed in the AAV-FIB-ADNF treated group. Thus, striatal infusion of AAV-FIB-ADNF protected substantia nigra TH containing neurons from 6-OHDA-induced cell death, a finding likely attributible to retrograde transport of the AAV-FIB-ADNF from the striatum to the substantia nigra. These findings illustrate the utility of AAV mediated expression and secretion of neuroprotective gene products *in viv*o.

The University of North Carolina has filed a patent application for the FIB secretory sequence in the names of Drs. Haberman and McCown.

GENE REGULATION: STEM CELLS

51. A Novel Approach to Stem Cell Gene Therapy: Controlling Stem Cell Differentiation with Engineered Zinc-Finger Protein Transcription Factors

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Stem cells hold tremendous promise for gene therapy, tissue engineering, and the treatment of a diverse range of injuries and disease. However, exploiting the full potential of these pluripotent progenitor cells requires the establishment of a new set of tools capable of controlling the molecular decisions that determine whether, and how these cells differentiate. Critical transitions in stem cells are controlled via signaling pathways and subsequent transcriptional regulation. In this regard, we have previously shown that designed zinc-finger protein transcription factors (ZFP TFs) are capable of regulating the expression of targeted endogenous genes with singular specificity. In the present study, we evaluated the ability of such designed ZFP TFs to control the differentiation of embryonic stem (ES) cells. To this end, we constructed ZFP TFs that target the mouse OCT4 gene, a major regulator of ES cell pluripotency and self-renewal. We show here that introduction of an activator version of this ZFP TF resulted in increased OCT4 mRNA, and the expected concomitant effects on the expression of OCT4 responsive